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NUTRITION REVIEWS

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MAY 29 1950

NUTRITION REVIEWS

VOL. 8 MAY, 1950 No. 5

IODINE—A FOOD ESSENTIAL

Iodine in appropriate amounts, like iron, calcium, copper, and many other chemical elements, is essential throughout life. Yet many people do not get enough iodine from their food to meet their normal requirements, and long-continued iodine deficiency may result in serious disease, or at least in a chronic state of borderline malnutrition.

It has been demonstrated many times in this country and abroad that iodine deficiency is easily corrected and better health achieved through the daily routine use of table salt to which small amounts of iodine have been added by the manufacturer. This salt is as pure as, and tastes no different from, ordinary salt. It can be obtained at no extra cost or inconvenience to the consumer, and he can voluntarily insure his supply of this food essential.

IODINE AND THE THYROID GLAND

The thyroid gland is a large U-shaped gland in the neck. It has been poetically described as contributing to the beauty of the neck by filling up the vacant spaces around the larynx, particularly in women. The weight of the thyroid gland in the male varies from 20 to 60 g., or about 1 to 2 ounces. The normal thyroid in women usually is a little heavier. Its rich supply of blood and lymph attests to the importance of this gland to the entire body.

Iodine taken in the form of iodized salt, or iodine-rich food, such as oysters, salmon, or tuna fish, travels through the blood in the form of a salt, an iodide. The thyroid gland, of all the body tissues, has an affinity for iodine. When the thyroid gland absorbs the iodide it is oxidized by an enzymatic process. This releases iodine in a form which readily combines with an amino acid, tyrosine. Two molecules of this compound combine to form thyroxine, which is the thyroid hormone. Thyroxine may be carried by the blood stream to produce striking effects in many parts of the body, or it may be stored as a colloid, thyroglobulin, to be called upon when needed.

COURSE OF FOOD IODINE IN THE BODY

Food iodine	iodized salt, seafood
↓	
Alimentary canal	iodine absorbed by body
↓	
Blood stream	iodine carried to the thyroid
↓	
Thyroid gland	iodide (iodine) + tyrosine = thyroxine + protein = thyroglobulin
↓	
Thyroglobulin	used as hormone or stored as colloid

Just as calcium is essential for formation of bones, and iron is necessary to form hemoglobin for blood, so iodine is absolutely essential for formation of thyroxine. The thyroid normally contains from 15 to 20 mg. of iodine. In normal adults a supply of about 75 mg. of iodine per year will maintain the thyroid in normal condition.

The thyroid hormone controls the rate of heat production in the body by controlling the rate of cell oxidation. Heat production is coupled with energy liberation from the cells. Basal metabolism tests measure thyroid activity by measuring the rate of heat production in a resting individual.

Other functions of the thyroid are to aid in stimulation of normal growth of bones, hair, and skin; normal development of the brain; stimulation of sexual development at puberty; maintenance of a normal pregnancy; and production of an adequate milk supply during nursing.

When the thyroid becomes enlarged, it is recognized as a goiter.

Simple goiter. This type of goiter is by far

the most common form. It begins with a lessening in the amount of iodine in the colloid (the storage fluid in the thyroid gland) and an increase in size and number of thyroid cells. The enlargement which occurs in simple goiter may be accompanied by a mild degree of hypothyroidism, or underactivity. The enlargement represents an effort to manufacture more adequate amounts of thyroxine.

Simple goiter is so called because it does not cause any toxic or poisoning symptoms. Its importance from a medical standpoint is due to the fact that it may lead to a more serious form and may subsequently become toxic or poisonous; also, symptoms due to pressure on neighboring structures such as the windpipe may occasionally be bothersome. Where iodized salt is used from infancy, a simple goiter, with very few exceptions, is avoided.

Cretinism. A cretin is a child who is dwarfed by lack of sufficient thyroid secretion during fetal life. Cretins, in the past, were found in districts where goiter was common. Among these areas are the Himalayas, the Pyrenees, the Alps, the Andes in South America, the St. Lawrence and Great Lakes regions, and the northwestern area of the United States. So many of these unfortunates were found in certain localities in the Alps that government aid was necessary to support them.

In cretins, the mental, physical, and sexual development is greatly retarded. If these cretin dwarfs live to adulthood, they retain their childhood body build, and may not mature sexually. If untreated, their mentality may be arrested at a low level, making them unable to support themselves. The metabolism is very low, the skin has a typical dry, thick appearance, and deafmutism is common. At autopsy, abnormal or very small thyroid glands are found.

If thyroid extract is given to cretins at an early age, marked improvements result but complete recovery does not always occur. Cretinism should be prevented by assuring an adequate supply of iodine for the mother before and during pregnancy.

Myxedema. When destruction or degeneration of the thyroid gland occurs at any time after birth, the hypothyroid condition called myxedema results. Typical manifestations are a loss of mental and physical vigor, dry brittle hair, an apathetic, lethargic reaction to mental stimuli, a peculiar thickening of the skin, and a low-metabolic rate.

The outlook for the adult with myxedema is hopeful today. Three months of administration of thyroid extract makes a dramatic improvement. Thyroid extract will control the symptoms of myxedema entirely, and keep the patient in good health.

Hyperthyroidism. Hyperthyroidism, or toxic goiter, may begin in a previously healthy individual with enlargement of the gland and simultaneous toxic symptoms of rapid pulse, palpitation, tremor, nervousness, restlessness, and irritability. The type of toxic goiter is called primary or exophthalmic goiter, or Graves' disease.

A severe, primary, toxic goiter may soon be accompanied by a popeyed condition, medically called exophthalmos. The metabolic rate goes up, perhaps as high as 80 per cent or more above normal, and a rapid burning of fuel is accompanied by a rapid loss in weight. The animation increases to an abnormal nervousness which is very trying to the patient and his associates. There may be difficulty in breathing upon exertion and severe heart symptoms. Fortunately, various methods of cure for exophthalmic goiter are known today.

A temporarily increased demand for iodine may make a previously sufficient iodine supply dangerously inadequate. The well-known situations of increased demand are puberty, pregnancy, and lactation. Since the thyroid hormone is closely associated with the gonads, or sex glands, it is natural that sexual development should make an increased call for thyroid hormone. Enlargement of the thyroid often occurs during these growth periods. Many obstetricians

today are giving additional iodine to all their pregnant and lactating patients.

The Food and Nutrition Board of the National Research Council gives the daily requirement of iodine for an adult as 0.15 to 0.30 mg. and states that this need can be met by the regular use of iodized salt. Attention is called to the special importance of its use in adolescence and pregnancy.

Incidence of Goiter and Relation to Iodine

Goiter occurs throughout the world, wherever the supply of iodine is inadequate. Michigan lies in the Great Lakes goiter belt. In 1924 a survey placed the incidence of goiter in Michigan at 38.6 per cent. A promotion plan for consumption of iodized salt was put into effect, and in 1928 a re-examination showed the incidence to have decreased to 9 per cent.

During the depression approximately two-thirds of the families in the copper-mining community of Calumet, Michigan, were on relief. Relief officials, endeavoring to cut costs, distributed noniodized salt. The result was an upshoot in the goiter rate among the families using the iodine-lacking salt. Sixty per cent of the children who received noniodized salt had goiter. Only 3 per cent of the children, in the same community, who had remained on iodized salt had goiter.

Some plan of giving everyone the food equivalent of iodine has been tried in many parts of the globe for many years. The most popular and most practical way has been to add an infinitesimal (0.01 per cent) amount of necessary iodine to table salt.

Since iodine occurs in nature in some sources of salt, iodized salt is properly regarded as a natural food. It is an improved salt. Simple goiter may be prevented by eating food iodine, and tiny preventive amounts of food iodine in iodized salt are safe for the sufferer from toxic goiter. Many people who take vitamin tablets and are careful about getting an adequate supply of vitamins neglect the need for a normal supply of iodine. With today's emphasis on positive health, many medical authorities are actively endorsing the use of food iodine as a simple, cheap, easy insurance against the possible handicaps of a subclinical iodine deficiency.

Why Iodine May Be Inadequate in Diets

From ancient times goiter has been known to exist in certain regions. In areas where endemic goiter exists, the soil, water, and vegetation are poor in iodine. Analysis of drinking water in cities in the United States shows a wide variation in iodine content, from 0.01 microgram per kilogram in Duluth, Minnesota and Spokane, Washington to 73.30 micrograms per kilogram in San Dimas, California.

Seafood is a good source of iodine, but the iodine content varies with the variety of fish or crustacean and with the iodine content of the sea water from which it comes. Seafood-eating people, even in a goitrous area, are remarkably free from goiter.

Some natural salt deposits contain iodine, but others do not. A dramatic demonstration of man's accidental conversion of a naturally healthy area into a goitrous area occurred in the Kanawha River Valley of West Virginia. Prior to 1900, goiter was exceedingly rare, according to local physicians. Table salt came from the local salt wells. It was a crude, coarse salt with brown particles. After 1900, a sparkling white salt was shipped in. This white salt contained no iodine, but it pushed the crude salt off the market. During the next quarter of a century, the goiter rate rose sharply, and by 1922 a goiter survey showed that about 60 per cent of adolescent girls in that valley had goiter.

The iodine content of plants can be increased by addition of iodine-containing salts to the soil, and the iodine content of milk can also be increased by feeding suitable rations to the cow. However, such practices are considered to be utterly impractical in

meeting the goiter problem. They are too inefficient, uneconomic, and difficult to put into operation on a national scale.

It seems that the goiter problem in the United States today is due largely to lack of measures which would ensure everyone obtaining iodized salt. Many people do not recognize the importance of iodized salt, others have been misled into thinking it a medicated product, and representatives of the salt industry insist on making both iodized and uniodized salt available for household use. Legislation to correct this has been proposed, but is being held in abeyance until the effect of a new public health education campaign on iodized salt has been demonstrated.

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GROWTH FACTORS FOR BACTERIA

Previous reviews have noted the diverse nutritional requirements of various microorganisms (*Nutrition Reviews* **5,** *11, 154, 215, 219 (1947)*; **6,** *245 (1948)*). Among the bacterial growth stimulants, strepogenin has been particularly intriguing because of its apparent peptide nature and because it appeared to be active for mice as well as bacteria (*Ibid.* **4,** *273 (1946)*; **5,** *218 (1947)*; **6,** *223, 277 (1948)*). The identity of strepogenin is unknown, but D. W. Woolley has shown that L-serylglycyl-L-glutamic acid has activity for *Lactobacillus casei*, although it is less potent than the natural material.

In 1944, F. W. Chattaway, F. C. Happold, and M. Sandford (*Biochem. J.* **38,** *111 (1944)*) reported that an extract of liver was required in addition to the usual amino acids and other known nutrients for growth of *Corynebacterium diphtheriae gravis*. The active principle appeared to be an amino acid or small peptide because it was dialyzable, resistant to pepsin and trypsin, and inactivated by ninhydrin. More recently, the same or a similar material has been obtained in crude form from brewers' yeast (Chattaway, D. E. Dolby, D. A. Hall, and Happold, *Ibid.* **45,** *592 (1949)*). The product, Y47, active also for *Streptococcus faecalis R* and *L. casei*, was a mixture of carbohydrate and nitrogenous components. Its potency was due to the latter, inasmuch as treatment with nitrous acid resulted in inactivation. It was not adsorbed on norit although the liver fraction studied previously was readily adsorbed. Histidine, tyrosine, arginine, tryptophan, sulfur amino acids, and free amines were absent. Two-dimensional chromatography (*Nutrition Reviews* **7,** *195 (1949)*) disclosed a total of twelve constituents of amino acid or peptide nature. Five of these were identified as serine, glycine, isoleucine, aspartic acid, and glutamic acid. Four of the remainder were peptides since they disappeared from the chromatograms after acid hydrolysis of the preparation. Three were either amino acids or very acid-resistant peptides.

Most of the activity of Y47 resided in four fractions, two peptides, P1 and P2, and two acid-stable forms, S1 and S2. Neither S1 nor S2 could be correlated with known amino acids. Peptide P2 was more easily hydrolyzed than P1 and it appeared that the latter might be a hydrolytic product of P2. Complete hydrolysis of P2 yielded serine, glycine, and glutamic acid. Whether the structural arrangement of these three acids in P2 was the same as that in the tripeptide studied by Woolley was not determined, but the similarity is striking. Several dipeptides were synthesized in an effort to duplicate the activity of P1. Of these, glutamylglutamic acid, glutamylglycine, and glycylglutamic acid were moderately effective.

The data of Chattaway *et al.* may be considered additional evidence for the im-

portance of one or more simple peptides or new amino acids in the nutrition of *C. diphtheriae*. Although all four fractions appeared to be necessary for maximum growth, this conclusion may be modified later by quantitative studies on pure compounds. The relation of these substances in liver and yeast to other nutrients for bacteria, folic acid, or vitamin B_{12} for instance, must await determination of structure and function.

RIBOFLAVIN DEFICIENCY IN MAN

The signs and symptoms which may properly be attributed to a deficiency of riboflavin in man have been the subject of continuing controversy. Study of naturally occurring malnutrition seems to have brought little uniformity of opinion. Lack of suitable criteria to prove the nutritional origin of given lesions, difficulty in evaluating the separate influence of various vitamins in patients with multiple deficiency, and the widely varying nutritional background of patients used in various studies have compounded these difficulties.

One would expect that this situation might be clarified most easily by studying pure riboflavin deficiency produced experimentally in man under carefully controlled conditions. However, since the original description of experimental riboflavin deficiency in man (W. H. Sebrell and R. E. Butler, *Pub. Health Reports* **53,** *2282 (1938)*; **54,** *2121 (1939)*), other investigators (*Nutrition Reviews* **3,** *235 (1945)*; **4,** *130 (1946)*; **5,** *60 (1947)*) in several experiments have failed to reproduce the clinical picture observed by Sebrell and Butler. These other experiments differed in important respects in that either the duration of the experiment was shorter or the allowance of riboflavin was greater than that used by Sebrell and Butler.

It is of considerable interest, therefore, to note the report of M. K. Horwitt *et al.* (*J. Nutrition* **39,** *357 (1949)*) who have been able to produce clinical ariboflavinosis closely resembling that described by Sebrell and Butler. Their experimental subjects were inmates of a mental hospital, carefully selected as to physical condition, eating habits, emotional stability, and cooperativeness. The psychiatric diagnoses varied widely to minimize the chance that a metabolic defect involving riboflavin might be associated with the mental disease.

After a trial period of three months using a diet containing 1.1 mg. of riboflavin but similar otherwise to the diets used later, 39 subjects were selected and placed into three groups. Group A, 15 male subjects, received a diet composed principally of natural foods which supplied 0.55 mg. of riboflavin (9.0 micrograms per kilogram of body weight per day), 57 g. of protein, and 65 g. of fat in 2200 calories daily. Group B, 14 subjects, received the same diet but in addition were given a tablet containing 2 mg. of riboflavin each day. Both of these groups received supplemental vitamins (except riboflavin), calcium, and iron to assure adequate intake of essentials other than the vitamin under study. Group C, 10 subjects, ate the regular hospital diet ad libitum. The riboflavin content of all diets was checked continuously using both fluorometric and microbiologic methods during the first year of the study. The patients were followed with a large battery of clinical and laboratory examinations.

In the riboflavin-restricted Group A, the first clinical manifestation of a possible riboflavin deficiency appeared four months after beginning the diet in a patient who developed a typical herpes simplex vesicle at the left oral angle. During the next six weeks, horizontal fissures with crusting appeared at both oral angles. Local, nonspecific therapy slowly healed the lesions on the right. The lesion on the left did not heal completely until riboflavin was given. This patient also had seborrheic lesions on the scalp and hairy

areas of the chest, severe vertical fissuring at the nasolabial folds which eroded through the mucocutaneous border into the nostrils, and small fissures at the nasal orifice. All of these conditions healed rapidly after administration of riboflavin.

A second patient, ten months on the diet, developed similar lesions without appearance of pre-existing herpes. This patient also had a severe scrotal dermatitis which necessitated riboflavin therapy. Six milligrams of riboflavin per day produced marked improvement in forty-eight hours and complete healing in two weeks. This patient did not consume all of his diet and so had a riboflavin intake of only 0.44 mg. per day.

A third subject developed an angular fissure which persisted for five weeks but healed without therapy or recurrence. Cheilosis was seen in only one patient and the lesion was never more than minimum.

The most prevalent clinical lesion observed in these patients was scrotal dermatitis. In Group A, 12 of the 15 subjects developed mild to severe forms of this condition between the eighth and tenth month on the diet. The scrotum was markedly reddened with scaling and desquamation of the superficial skin layers. The lesions were discrete, bilateral, and not connected in the midline. In its most severe form the scrotal surface was raw and weeping and the process extended to adjacent areas of the thighs. These severe lesions did not heal spontaneously or with local saline applications but did heal dramatically when riboflavin was administered.

After all of these lesions had appeared in Group A, 3 subjects in Group B developed scrotal erythema with little or no scaling. This led to an examination of the general hospital population. Scrotal erythema, but no scaling, was found in a large percentage of those who were incontinent. Thus it was evident that these scrotal changes were not a pure manifestation of riboflavin deficiency. On the other hand, it seemed likely that riboflavin deficiency greatly increased the severity and perhaps the incidence of the lesions.

Scrotal dermatitis does not seem to have been reported previously as a sign of riboflavin deficiency. However, R. J. Vakil (*Nutrition Reviews* **4,** *319 (1946)*) reported one patient thought to be riboflavin deficient who showed a vulvar dermatitis which responded rapidly to riboflavin. G. H. Berryman and co-workers (*Am. J. Physiol.* **148,** *618 (1947)*) observed scrotal dermatitis in severe vitamin B-complex deficiency. J. Goldberger and G. A. Wheeler (*U.S.P.H.S. Hygienic Lab. Bull.* **120,** *7 (1920)*) observed scrotal dermatitis frequently in their pellagrins although others have seen it much less commonly (S. Harris, "Clinical Pellagra," *p. 313, C. V. Mosby Co., St. Louis (1941)*). Perhaps the lesion in these cases may have been due, in part at least, to concomitant riboflavin deficiency.

Of the other tests employed, only flicker fusion and urinary riboflavin excretion were abnormal. All but one of the patients in Group A showed an increase in the threshold for flicker fusion when compared with the subjects in the riboflavin-supplemented group. The urinary excretion of riboflavin dropped from 400 micrograms in twenty-four hours on the hospital diet (1.6 mg. of riboflavin per day) to 112 micrograms on the 1.1 mg. riboflavin-pre-experimental diet. Seven days after the 0.55 mg. diet was started the average excretion dropped to 50 micrograms and remained at about that level for nine months, then fell to as little as 36 micrograms per day. When 2 mg. of riboflavin were given once, twice, or three times a day to depleted subjects, tissue saturation, as determined by the four-hour excretion test, was obtained in thirty-eight, twenty-five, and eleven days, respectively. Blood glucose, pyruvate, and lactate determinations revealed no evidence of disturbed carbohydrate metabolism. Hematologic examinations and liver function tests gave results within the normal range. Electrocardiograms, heart orthodiagrams, and basal

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metabolic rates were normal. The extensive neurologic and psychiatric testing of these patients will apparently be reported elsewhere. The authors do not mention whether corneal vascularization was observed.

The deficient diet used in the clinical study was fed to weanling rats with and without riboflavin supplementation to demonstrate that the diet was also deficient for the rat. While their growth curves clearly support the fact that this diet was suboptimal in riboflavin, it is also evident that only a mild deficiency was produced when compared with the usual riboflavin deficiency produced in rats in the experimental laboratory. Their deficient animals apparently survived and continued to gain some weight for more than a hundred and sixty days, attaining body weights of 250 g. If man could be expected to respond as do rats, these results provide a ready explanation for failure of more of the patients to develop more severe lesions.

The authors interpret their results as confirming the report of Sebrell and Butler (*loc. cit.*) that dermatologic signs of riboflavin deficiency can be produced at a riboflavin level of 0.5 mg. per day. The earlier failure by M. K. Horwitt, E. Liebert, O. Kreisler, and P. Wittman (*National Research Council Bull.* **116** (*1948*)) to produce riboflavin deficiency with daily riboflavin intakes of 0.8 to 0.9 mg. over a period of two years, the negative results of other workers using daily riboflavin allowances in the same range (R. D. Williams, H. L. Mason, P. L. Cusick, and R. M. Wilder, *J. Nutrition* **25,** *361* (*1943*); A. Keys *et al.*, *Ibid.* **27,** *165* (*1944*)), and the positive results of the present study with 0.55 mg. per day, make it probable that 0.5 mg. of riboflavin per day is about the critical level at which most patients can be expected to develop recognizable riboflavin deficiency within several months.

The study of Horwitt and associates does not permit definite conclusions as to the specificity of the various clinical lesions observed. In their experiments the appearance of local lesions was usually preceded by lesions of nonspecific or bacterial origin which failed to heal, presumably due to lack of riboflavin. Thus, the scrotal dermatitis seemed conditioned by pre-existing nonspecific irritation, some of the angular stomatitis by herpes, and the lesions around the nose by an upper respiratory infection. However, all of these lesions cleared remarkably when riboflavin was given. In their view then, the lesions observed in riboflavin deficiency may depend not only on the dietary riboflavin and the length of the depletion period, but also on the presence or absence and location of initial trauma.

This idea is not new but is most difficult to prove with certainty. Their experiments do provide fresh support for this possibility. Horwitt *et al.* performed wounding experiments in riboflavin-deficient rats which demonstrated that such animals repair wounds very slowly, in confirmation of the results of M. D. Bosse and A. E. Axelrod (*Proc. Soc. Exp. Biol. Med.* **67,** *418* (*1948*)).

From the clinical results and from the urinary excretion data, Horwitt and coworkers estimate the basal riboflavin requirement of an adult at 1.1 to 1.6 mg. per day. Proper provision for emergency requirements such as illness and tissue repair should increase these values somewhat. These figures fit well with those recommended by the Food and Nutrition Board of the National Research Council (*Nutrition Reviews* **6,** *319* (*1948*)).

VITAMIN E AND TISSUE METABOLISM *IN VITRO*

A specific role for vitamin E in tissue metabolism has not yet been found, despite numerous studies of the biochemistry of tissues from vitamin E-deficient animals (*Nutrition Reviews* **6,** *346* (*1948*)). At present, only its antioxidant properties allow one to

assign to it the fairly nonspecific role of guardianship over easily oxidized vitamins and accessory factors in transit or in storage (K. C. D. Hickman and P. L. Harris, *Advances in Enzymology* **6,** *469 (1946)*).

The action of alpha-tocopherol phosphate in reducing the heightened oxygen consumption of dystrophic muscle from vitamin E-deficient animals, both *in vivo* and *in vitro* (O. B. Houchin and H. A. Mattill, *J. Biol. Chem.* **146,** *309 (1942)*), has led to the conclusion that the phosphate of vitamin E is its physiologic form, particularly since it is well established that phosphorylation is important in conferring coenzyme activity upon other vitamins such as thiamine and pyridoxine. Actually, however, there is little evidence to support the assumption that alpha-tocopherol phosphate is the active form of vitamin E in the body.

Despite the observation of P. Karrer and G. Bussmann (*Helvet. chim. acta* **23,** *1137 (1940)*) that alpha-tocopherol phosphate was more active than the free alcohol in biologic tests of vitamin E activity, and the suggestion of S. Morgulis and H. P. Jacobi (*Quart. Bull. Northwestern Univ. Med. School* **20,** *92 (1946)*) that the phosphate bond of alpha-tocopherol phosphate may be of the high-energy type found in adenosinetriphosphate, no one has isolated alpha-tocopherol phosphate from tissue or shown it to be specifically active in a purified enzyme system. Furthermore, alpha-tocopherol phosphate is neither an antioxidant nor a member of any oxidation-reduction system.

W. M. Govier, V. Bergmann, and K. H. Beyer (*J. Pharmacol. Exp. Therap.* **85,** *143 (1945)*) explained the action of alpha-tocopherol phosphate on succinoxidase by postulating an inhibitory effect on DPN-ase, the enzyme which destroys diphosphopyridinenucleotide (DPN). The preservation of DPN was thought then to facilitate the oxidation of malate to oxalacetate which is a potent inhibitor of succinic acid dehydrogenase. This explanation was questioned by S. R. Ames (*J. Biol. Chem.* **169,** *503 (1947)*) who showed that alpha-tocopherol phosphate precipitates ionic calcium from solution. Since calcium serves as an activator for DPN-ase, part of the effect of alpha-tocopherol phosphate on succinoxidase could be attributed to an indirect effect of the phosphorylated vitamin.

More recently, S. R. Ames and H. A. Risley (*Ann. N. Y. Acad. Sci.* **52,** *149 (1949)*) have shown that the effect of alpha-tocopherol phosphate upon succinoxidase may be more directly associated with an effect upon enzyme protein, but at the same time more distantly related to the biologic role of vitamin E. These workers compared the effects of alpha-, gamma-, and delta-tocopherol phosphates upon succinoxidase activity *in vitro* and concluded that all esters inhibited the enzyme complex to the same extent and all inhibitions were released by addition of calcium ions to the same extent. Since alpha-, gamma-, and delta-tocopherols vary widely in biologic vitamin E potency, it was concluded that the effects of their phosphates *in vitro* bore no relation to the biologic action of the vitamin. That combination between these phosphates and the proteins of the succinoxidase complex in all probability occurred, was indicated by the additional finding of these workers that the intrinsic viscosity of crystalline bovine serum albumin increased markedly upon addition of tocopherol phosphate.

In related studies, P. D. Boyer, M. Rabinovitz, and E. Liebe (*Ann. N. Y. Acad. Sci.* **52,** *188 (1949)*) found that alpha-tocopherol phosphate and dodecyl sulfate, another anionic compound with a large nonpolar group, both inhibited the succinoxidase activity of tissue homogenates. This inhibitory action could be prevented by prior addition of serum albumin to the homogenates. Other surface-active agents were found to act similarly and it was concluded that the effects of alpha-tocopherol phosphate on succinoxidase were nonspecific.

In an attempt to localize the site of action of these agents on the succinoxidase system

it was demonstrated that (1) accumulation of oxalacetic acid arising from the protective action of alpha-tocopherol phosphate upon DPN-ase was not a significant factor in the inhibition; (2) succinic acid dehydrogenase, as shown by methylene-blue reduction, was still active in the inhibited preparation; (3) the cytochrome-oxidase component, as shown by oxidation of ascorbate, was only partially inhibited in the totally inhibited system; and (4) addition of cytochrome-c did not reverse the inhibition.

On the basis of these findings, Boyer *et al.* concluded that the probable site of interference by alpha-tocopherol phosphate and related compounds in this system was the point of coupling between the dehydrogenase and the iron-porphyrin enzymes.

Other studies with alpha-tocopherol phosphate deserve mention. In 1948, J. P. Hummel (*J. Biol. Chem.* **172,** *421 (1948)*) called attention to the fact that homogenates of dystrophic muscle from vitamin E-deficient animals showed a reduced phosphorylation of creatine when compared with normals, but was unable to demonstrate increases with added alpha-tocopherol phosphate.

W. M. Govier and A. J. Gibbons (*Ann. N. Y. Acad. Sci.* **52,** *163 (1949)*) have confirmed this finding with homogenates of cardiac muscle from vitamin E-deficient guinea pigs, and were able to demonstrate slight increases in phosphorylation of creatine upon addition of alpha-tocopherol phosphate which, however, were abolished by simultaneous addition of potassium ions. Although the finding of decreased high-energy phosphorylation in vitamin E-deficient tissue may be a clue to the action of vitamin E *in vivo*, the action of alpha-tocopherol phosphate on phosphorylation *in vitro* must be reexamined for specificity.

In addition to changes in the oxidative metabolism of dystrophic muscle from vitamin E-deficient animals, changes in amino acid metabolism occur. C. E. Roderuck, D. H. Basinski, and M. A. Barber (*Ann. N. Y. Acad. Sci.* **52,** *156 (1949)*) have studied transamination in homogenates of skeletal muscle from vitamin E-deficient rabbits and guinea pigs and found that transfer of an amino group from aspartic acid to alpha-ketoglutaric acid occurred only about 50 per cent as rapidly in the dystrophic tissue as in normal control muscle. Addition of pyridoxal phosphate had no effect upon this difference. Removal of the oxalacetic acid formed in the above reaction was not significantly different in deficient and normal specimens.

In addition to the defect in transamination, free-amino acid stores appear to be decreased in vitamin E-deficient muscle. Roderuck *et al.* found glutamine to be decreased to 30 per cent of normal in dystrophic muscle from vitamin E-deficient guinea pigs. Aminoaciduria is a feature of the dystrophy of vitamin E deficiency in rabbits as well as of progressive muscular dystrophy in man (S. R. Ames and H. A. Risley, *Proc. Soc. Exp. Biol. Med.* **68,** *131 (1948)*).

In attempting to solve the enigma of the action of vitamin E *in vivo* (by *in vitro* studies), it must be remembered that rigorous controls are required. Despite the difficulties, it is probable that a well-controlled study of the type described above, will eventually produce a definitive answer to the problem.

CALORIES AND THE POSTOPERATIVE NITROGEN DEFICIT

Excessive urinary nitrogen excretion has been observed following such damaging episodes as thermal burns, fractures, operations, and severe infections. This reaction appears to occur only in well-nourished individuals, nitrogen excretion remaining normal or low following similar traumatic episodes in those who are undernourished. Attempts, only oc-

casionally successful, have been made to overcome this nitrogen loss either by high-caloric or, more commonly, by high-protein feeding. These matters have been repeatedly discussed in this journal (*Nutrition Reviews* **3,** *40, 86, 253 (1945)*; **4,** *6 (1946)*; **5,** *46 (1947)*; **6,** *20, 161 (1948)*; **7,** *123 (1949)*). Some, but by no means all, of this so-called catabolic response following trauma may be laid to immobilization (*Ibid.* **6,** *146 (1948)*).

Following the period of catabolism of variable length an anabolic period appears during which nitrogen balance is positive.

It has been postulated that elaboration of an antianabolic hormone from the adrenal cortex is stimulated by the trauma which prevents anabolism of amino acids which are deaminized and the residue, at least in part, is available as carbohydrate. It has been further suggested that the anabolic period is characterized by production from the adrenal cortex of an anabolic "N" hormone.

S. C. Werner, D. V. Habif, H. T. Randall, and J. S. Lockwood (*Ann. Surg.* **130,** *688 (1949)*) have studied this problem of the postoperative nitrogen loss under controlled conditions, particularly with regard to the caloric intake. In a previous study in normal individuals, Werner (*J. Clin. Invest.* **27,** *561 (1948)*) demonstrated that changes in nitrogen excretion comparable to those seen after traumatic occurrences could be observed following only reduction in caloric intake.

Proceeding from these studies in normal individuals, 26 patients undergoing operation (cholecystectomy, hernia repair, colotomy) were studied in four groups. It is stated that "all patients were in good health and nutrition, and previously active." Inasmuch as the study was fundamentally a nutritional one, better definition of the nutritional status of the individuals would have added strength to the paper. The protein intake lay between 12.5 and 14.4 g. of nitrogen per day and in each individual was maintained constant throughout the entire pre- and postoperative periods of study. All four groups were studied in the first period of one week's duration during which time they received between 30 and 40 calories per kilogram of body weight. In the first two groups of patients the caloric intake was reduced for the next week, which was immediately preoperative, to between 18 and 24 calories per kilogram. The same feeding program was continued for four days postoperatively and then the calories were returned to the higher intake for three days. These two groups (I and II) were thus comparable in caloric intake but feeding was by mouth in Group I, whereas for three days preoperative and four days postoperative all feeding was intravenous in Group II.

In a third group on parenteral administration, caloric intake was reduced for four days, raised a week before the operation, and reduced again immediately following operation. The final group was maintained on the higher caloric intake, presumably by mouth, for a week before and ten days after the operation. There were 5, 12, 3, and 6 patients in Groups I, II, III, and IV, respectively.

The results are expressed in averages for each group of patients in each period. Nitrogen equilibrium or a slight positive nitrogen balance was the average finding in all periods in the fourth group maintained throughout on a high-caloric intake by mouth. In all the other groups the average nitrogen balance was negative throughout except in the first group in which approximate equilibrium was maintained for the brief period of three days just prior to the operation.

The authors interpret these results to indicate that when the caloric and protein content of the diet is maintained before, during, and after the operation negative nitrogen balance does not occur, but that it is regularly seen when the calories are reduced either before or after the operation. They further point out that a negative nitrogen balance which increased postoperatively was seen only when the caloric intake was low. The type of surgical procedure

appeared to have no influence upon the outcome so far as nitrogen balance was concerned.

Although the change from oral to intravenous feeding did not change the average nitrogen balance, 5 of the 12 patients in whom this shift in route of administration was undertaken did show some increase in negative nitrogen balance following the change.

The authors conclude from their data that "no loss of nitrogen is found following a surgical procedure beyond that observed on the same caloric intake before the operation." It is difficult to harmonize these results with the large body of work which was done before, to a large extent during the last war, indicating that catabolism of protein or antianabolism did occur following trauma, including operative procedures such as those reported here. The authors suggest that such factors as fever and infection may play a part but that the chief factor is too low a caloric intake. It is unfortunate that more patients were not studied in each group and that the periods of study were so short. Even a week seems a short period for a nutritional study of this type and certainly three or four days is too short to allow firm interpretation of the results. It is certainly possible that a simple caloric lack, as the authors say, may explain the increased nitrogen excretion following operation in many instances but whether this is true in all instances and whether the catabolic postoperative period concept must be completely cast aside cannot be answered from the data in this study. Further work, including more patients and with longer periods of observation, is certainly needed.

GROWTH PROMOTION BY VITAMIN B_{12} IN CHILDREN

In clinical medicine vitamin, B_{12} has been regarded until now chiefly as the antipernicious anemia factor, whereas in animal husbandry its effect on growth and development has been fully recognized and applied in practice.

While growth promotion can be measured under closely controlled and uniform conditions in chicks and other small laboratory animals, similar comparable conditions are not available when it is desired to measure growth promotion in children.

Children are genetically different individuals who vary in their inherent growth characteristics, in contrast with chicks which may be produced in large numbers in genetically identical broods. Furthermore, changes in weight, size, and body conformation occur much more slowly in children than they do in chicks. Hence, day-to-day weight measurements which are valuable in studies on chicks are meaningless when used on children.

The task of reducing growth measurements in children to a scientifically comparable basis seems to have been approximated with considerable success by the graphic method known as the "Wetzel Grid Technique" (N. C. Wetzel, in "Medical Physics" (O. Glasser, Ed.), *Year Book Publishers Inc., Chicago (1944)*; "The Treatment of Growth Failure in Children," *NEA Service Inc., Cleveland (1948)*), in which two criteria become decisive, namely, the direction and the speed of physical development. For, a child is at once marked as a "growth failure" the moment it deviates beyond known tolerance limits of individual variation which in a Grid automatically allows for accidental fluctuations in measurements.

Technically, the Grid coordinates of direction are body size and shape (physique) as represented in a level-channel system, the location of a given point within this system being plotted from a corresponding pair of values of body weight and height. Successive points trace out a course of development whose direction may thus be measured

at sight by the fidelity with which a child's curve parallels its own channel. For every 10 levels increase in size a healthy child will maintain his physique to within ½ channel; put another way, the direction of growth under conditions of health is characterized in the Grid technic by changes in body size that always exceed the magnitude of changes in physique by a ratio of at least 10:1. In the particular type of growth failure that is associated with malnutrition a child does not maintain that ratio; it "cross-channels" with the result that it loses a corresponding amount of physique.

In somewhat similar fashion, developmental speed is measured by the fidelity with which a child keeps up with his own schedule of progress (auxodrome), regardless of age, physique, or degree of advancement with respect to the general population of children. The rule is that a child progress at the rate of 1 level line per month, or 12 levels per year, and accordingly, that he attain (within a level or so) a predeterminable year-end position as he "grows" older. Again, inability to do so marks him as a "growth failure" no matter whether he did or did not maintain proper direction in the channel system. Obviously, slow-down unaccompanied by channel change is not as serious as slow-down in rate which is accompanied by bodily changes sufficient to alter physique, especially in the direction of physique loss.

Through its channels, levels, and auxodromes the Grid technic thus makes it possible, with the help of ordinary weights and heights, to ascertain both physical status and growth progress in terms of size-shape-direction and speed. It furnished a direct method for evaluating child growth, specifically, whether this is or is not satisfactory, for the decision rests entirely upon the facts as to whether direction and speed have been maintained within the regular tolerance limits of ½ channel per 10 levels of advance, and at the rate of 1 level line per month, respectively. Yet, however important these parameters and their tolerances are, there is still another point that may be emphasized: for the values just given apply to all school-age children since they are independent of race, sex, weight, height, size, shape, and (up to maturation) of age. A single direction and a single speed thus become the standard of comparison by which the growth of individually different children may be measured, checked, and appraised as essentially "good" or "bad." One may, finally, compute level gains and speed to any desired degree of numerical accuracy. It should therefore be of interest to learn what an application of the Grid technic can reveal when vitamin B_{12} is administered to a particular group of children, as has recently been reported (N. C. Wetzel, W. C. Fargo, I. H. Smith, and J. Helikson, *Science* **110,** *651 (1949)*).

Since vitamin B_{12} could not be expected *a priori* to manifest an effect on human growth when administered orally in small doses (10 micrograms per day) to children who were "growing normally" according to the aforementioned Grid standards—even though it might actually be concerned with child growth,—the authors set out to ascertain whether B_{12} would reveal "growth-promoting" properties under the special circumstances of growth failure, in particular whether it would enhance the recovery of children who were under regular care for growth failure and its attending malnutrition at the year-around Fresh Air Camp, of Cleveland.

Eleven children (6 boys, 5 girls; ages 5 to 12 years) with moderate to severe malnutrition and simple growth failure were each given daily doses of 10 micrograms of crystalline vitamin B_{12} by mouth. Three of the treated group were selected owing to unaccountably slow recovery under routine care; 8 being chosen at random, from a resident population of 76, the 65 remaining children thus constituting the untreated time-place-season control group. Vitamin B_{12} was administered at the time of the usual midmorn-

ing milk offering; heights and weights were remeasured weekly at the same hour, and all routine procedures (rest, exercise, diet, etc.) were continued in usual fashion.

Before and after values as regards growth were measured by means of the Wetzel Grid technic, and thus in terms of the direction and speed of physical development, more specifically, in terms of physique loss or gain, level per month progress, and their standard errors, with allowance for degrees of freedom available from pooled before and after variances.

Five of the 11 subjects responded positively and definitely to B_{12} ingestion; and 4 of these made an average gain of more than 8 levels in eight weeks or about $4\frac{1}{2}$ levels per month as compared with their own average recovery rate before B_{12}, of 1.4 levels per month. The fifth child who had been developing at the rate of 1.113 ± 0.050 level per month during a previous two hundred and eight-day control period showed an *increase* of 2.06 ± 0.23 level per month, which, for 13 degrees of freedom, gives a t-value of 8.96! The authors state that p (the probability of the result being due to chance) $<$ (is less than) 0.001. In reality, the probability that such a positive increase over the control period rate is due to chance, is more nearly 1 in 2 billion! Since the "growth-promoting" effect of B_{12} in the case of the 5 reactors was thus beyond doubt, it may be presumed that their responses represented reactions to "functional" if not actual B_{12} deficiency which was thus improved by B_{12} ingestion. Conversely, it would appear that the 6 nonreactors were not demonstrably in need of B_{12}, since their gain was not significantly greater under treatment (2.00 ± 0.70 level per month as compared with 1.86 ± 0.12 level per month previously).

Although there were no "characteristic" or suggestive physical signs in the hair, skin, eyes, mouth, or nervous system, prior to B_{12} administration, the growth responses were, however, accompanied by manifestations of increased physical vigor, alertness, and greater appetite. Severe symptoms of allergic bronchitis in one boy were so greatly attenuated, that the authors suggest further investigation of the relations between B_{12}, protein metabolism, and allergic disturbances.

These experiments on the effect of crystalline B_{12} in growth failure of children have demonstrated that B_{12} has far more general nutritional significance than that represented by its antipernicious anemia properties. Vitamin B_{12}, in these studies, has been shown to have its general nutritional effects revealed in human beings by a method suited to the measurement of human growth, as something distinct from mere height or weight increments, for the Wetzel Grid technic, which was thus employed, provides standards of growth direction and speed that are independent of race, sex, weight, height, size, or shape, and (up to maturation) of age.

Obviously this small series of observations requires further confirmation and extension, including other age groups, with special reference to infants. Based on animal experiments, it may be expected that vitamin B_{12} will prove to be effective in various clinical conditions, such as fatty liver, protein deficiency, and thyrotoxicosis. Its practical value in human nutrition has still to be determined.

INCREASED VITAMIN E REQUIREMENT OF CHICKS IN HYPERTHYROIDISM

Within recent years it has been recognized that the increased rate of metabolism induced by administration of thyroid substance in animals results in an enhanced requirement not only for calories, but also for many of the vitamins required in metabolic reactions (*Nutrition Reviews* **6**, *54 (1948)*; **7**, *75, 183 (1949)*). Among the vita-

mins reported to be required in increased amounts by thyrotoxic animals are thiamine, vitamin B_6, pantothenic acid, vitamin A, ascorbic acid, and vitamin B_{12}. The pronounced effects of administration of the latter vitamin to animals rendered thyrotoxic by feeding of iodinated casein or thyroid substance have been noted previously, and form the basis for animal assay procedures for this vitamin (*Ibid.* **7,** *183, 210 (1949)*). Sufficient amounts of each of these known pure vitamins in an otherwise complete synthetic ration go far toward counteracting the deleterious effects of thyrotoxicosis in rats. Unidentified factors present in liver and other food sources are also required to overcome completely the depressing effects of thyroid administration upon growth rate. A study by R. S. Wheeler and J. D. Perkinson (*Am. J. Physiol.* **159,** *287 (1949)*) is of interest in this connection. These authors investigated the effects of hyper- and hypothyroidism on the vitamin E requirements of chicks. For this purpose, 200 male White Leghorn chicks, 4-days old, were divided at random into two groups and placed upon (a) a vitamin E-deficient ration, and (b) this same ration supplemented with alpha-tocopherol. Each of these groups was then subdivided into three subgroups as follows: (1) hyperthyroid group, in which the ration was supplemented with 0.04 per cent of thyroprotein; (2) untreated controls; and (3) hypothyroid group, in which the ration was supplemented with 0.1 per cent of thiouracil.

On the vitamin E-deficient ration, the hyperthyroid rats showed symptoms of vitamin E deficiency well before the untreated controls, and the latter in turn became deficient well before the hypothyroid rats. Forty-eight per cent of the hyperthyroid chicks were dead before any of the deficient controls had started to show deficiency symptoms, and 35 per cent of these deficient controls were dead by the time any of the hypothyroid chicks died. The mean age at death was 15, 20, and 28 days, respectively, for animals of these three subgroups, and the percentage mortality in these groups was 71, 53, and 41, respectively. All of the animals dying of vitamin E deficiency became ataxic and showed brain hemorrhages upon autopsy.

In the vitamin E-supplemented groups, on the other hand, none of the chicks showed symptoms of vitamin E deficiency, and the only deaths were among the hyperthyroid chicks. In this group there was only 15 per cent mortality, at an average age of 28 days, and these deaths appeared due to starvation rather than to vitamin E deficiency. Starvation seemed to result from a severe perosis (slipped tendon) which prevented the birds from obtaining access to food. It was not decided with certainty whether this perosis was a delayed symptom of vitamin E deficiency or whether it resulted from other deficiencies in the ration of these hyperthyroid chicks. Such a condition is known to result from manganese, biotin, and choline deficiencies.

Vitamin E must thus be added to the list of vitamins required in enhanced amounts by hyperthyroid animals. The observations have possible utility in development of a more rapid and accurate bioassay for vitamin E. They may also have clinical implications. Sufficient results with laboratory animals have now been reported to indicate that requirements for several vitamins are increased during hyperthyroidism, and that by supplying increased amounts of such nutritional factors required, many, if not all, of the deleterious effects of hyperthyroidism can be prevented. A new approach to management of the hyperthyroid patient may thus become possible.

EFFECT OF HYALURONIDASE ON ABSORPTION OF FLUIDS ADMINISTERED SUBCUTANEOUSLY

Hyaluronic acid is an important constituent of the ground substance of connective tissue. It is a mucopolysaccharide with the physical properties of a gel, tending to bind water in interstitial spaces and inhibiting diffusion in tissues. A mucolytic enzyme capable of depolymerization of hyaluronic acid has been isolated from testicular tissue and a number of other sources. The enzyme, hyaluronidase, has proved to be an effective tool in many types of biologic investigations (K. Meyer, *Physiol. Rev.* **27,** *335 (1947)*).

Action of hyaluronidase on the ground substance of connective tissue suggested that it might be clinically useful in enhancing the absorption of fluids administered subcutaneously. In dehydrated infants, children, and adults the problem of fluid administration is one which at times tests the ingenuity of the physician. If adequate quantities of fluid are to be administered within reasonable periods of time and without major discomfort to the patient, it is often necessary to approach the peripheral veins through surgical procedures. The latter procedure is sometimes fraught with technical difficulties.

L. S. Sannella (*Yale J. Biol. Med.* **12,** *433 (1940)*), proposed the use of hyaluronidase to facilitate absorption of subcutaneous fluids. In experimental animals he was able to prove that the enzyme enhanced fluid absorption and made it more rapid. The preparation of hyaluronidase used by Sannella was admittedly crude. Although no signs of toxicity were manifest, there were evidences of development of hypersensitivity to proteins in the enzyme preparation.

In the past several years hyaluronidase preparations of greater degrees of purity have become available and have been utilized in investigations appraising their adaptability for clinical use (O. Hechter, S. K. Dopkeen, and M. H. Yudell, *J. Pediat.* **30,** *645 (1947)*; L. C. Burket and P. György, *Pediatrics* **3,** *56 (1949)*). It has been clearly demonstrated that the rate of fluid absorption is increased and that the pain associated with tissue distention in hypodermoclysis is decreased when as small a quantity as 10 to 20 micrograms of hyaluronidase is injected into the site of infusion. The induration due to the subcutaneous injection of relatively large volumes of fluid disappeared within ten to twenty minutes in enzyme-treated areas, whereas sixty to ninety minutes were required for absorption of the same volumes of fluid when hyaluronidase was not used. There were neither toxic reactions nor evidences of sensitization to the foreign protein in the small doses of enzymes required to produce an enhancing effect of fluid absorption. In the quantities administered, hyaluronidase did not accentuate pre-existing infections nor did it produce changes in renal or hepatic function.

Further evaluations of hyaluronidase and its adaptation for clinical purposes are provided by J. Schwartzman and M. Levbarg (*J. Pediat.* **36,** *79 (1950)*). These authors have attempted to determine the optimum quantity of the enzyme to be used in facilitating absorption of fluids. Two standard purified preparations were tested. Activities were expressed in viscosity units, the unit representing the amount of hyaluronidase required to produce 50 per cent reduction of viscosity of a standard solution of hyaluronic acid in thirty minutes. With one preparation the rate of absorption increased up to the level of 100 units. Injection of additional quantities of enzyme did not enhance this effect. With a second preparation, 500 viscosity units provided the maximal effect. The conclusion drawn was that standardization of hyaluronidase in terms of its *in vitro* effect on the viscosity of solutions of hyaluronic acid did not provide a quantitative measure of its effect *in vivo* on the absorption of fluids.

The ability of hyaluronidase to facilitate absorption of 250 to 300 ml. quantities of

electrolyte solutions injected subcutaneously into selected patients was studied. When enzyme was not added to the infusion, a period of a hundred and fifty to a hundred and eighty minutes was required to complete the injection and that tissue area could not be used again for hypodermoclysis until a period of about eight hours had elapsed. When hyaluronidase was administered with the fluid, the total volume was delivered completely into subcutaneous tissues in eighty minutes and other injections could be made into the same area soon thereafter. A single injection of enzyme maintained its effect for about five days. The authors demonstrated that hyaluronidase also was effective in enhancing the absorption of solutions containing glucose, calcium gluconate, protein hydrolysates, and various drugs. Plasma and whole blood were more rapidly absorbed from subcutaneous tissues when hyaluronidase was added.

The effectiveness of the enzyme was observed in patients with various diseases. One factor, alone, was found to inhibit or interfere. If the total proteins of plasma were less than 5.5 g. per 100 ml., no increase in the rate of absorption occurred when hyaluronidase was injected. Intravenous injection of plasma in quantities sufficient to correct preexisting hypoproteinemia made it possible to demonstrate the effectiveness of hyaluronidase in patients in whom it previously had no apparent action.

A somewhat more effective and quantitative measure of the influence of hyaluronidase in the absorption of fluid from tissues has been achieved by G. B. Forbes, R. W. Deisher, A. M. Perley, and A. F. Hartmann (*Science* **111,** *177 (1950)*). These investigators injected 0.5 ml. of isotonic saline solution containing 0.5 to 1.0 microcurie of radiosodium (Na^{24}) into the subcutaneous tissues of the forearm of a subject. The same solution plus 25 units of hyaluronidase was injected into the tissues of the opposite forearm. The disappearance rate of Na^{24} was determined by recording the gamma-ray activity at the site of injection at frequent intervals. Although rates of absorption without the enzyme showed considerable variation in 7 subjects, addition of hyaluronidase increased the rate of disappearance of Na^{24} in each instance. Fifty per cent of the radioactivity was lost after an average period of nine minutes without hyaluronidase. Presence of the enzyme reduced the time of 50 per cent disappearance to about five minutes. In most edematous patients studied, absorption of radiosodium was more rapid than normal and the rate was not accelerated by addition of hyaluronidase.

Forbes and associates (*loc. cit.*) were also able to demonstrate that radiosodium injected into the subcutaneous tissues was absorbed and appeared in the blood stream. Maximum concentrations of radiosodium in the serum were achieved sooner and the peak radioactivity was higher when hyaluronidase was included in the solution injected.

These reports present evidence that hyaluronidase may be utilized to facilitate administration of fluids given subcutaneously. Observations indicate that the quantities of enzyme injected for this purpose are not associated with toxic or side effects and that hyaluronidase may be employed with impunity. Restoration of favorable fluid or electrolyte balance may be a life-saving measure in severely dehydrated infants and children. Time is frequently lost because of slow rates of hypodermoclysis or because of difficulties met in attempting to effect intravenous infusion. Hyaluronidase would seem to present a new and important therapeutic adjunct which will be widely utilized in facilitating more rapid administration of solutions containing needed nutrients or drugs.

VITAMIN B_{12}, FOLIC ACID, AND CHOLINE INTERRELATIONS

In recent years it has become increasingly common to discover instances where a change in the amount of one dietary component profoundly changes the dietary requirement for another. Elucidation of these relations has generally led to important advances in knowledge of fundamental biochemistry and improvements in applied nutrition as well. The niacin-tryptophan and the methionine-cystine-choline relations are well-known examples.

There are many reasons to suspect that a relation might exist between folic acid, vitamin B_{12}, and choline. The partial interchangeability of vitamin B_{12} and methionine or choline in the diet of chicks and rats (H. R. Bird, M. Rubin, and A. C. Groschke, *J. Nutrition* **33,** *319 (1947)*; A. E. Schaefer, W. D. Salmon, and D. R. Strength, *Proc. Soc. Exp. Biol. Med.* **71,** *193, 202 (1949)*) and the similar action of folic acid, vitamin B_{12}, and choline in the treatment of certain macrocytic anemias in man (*Nutrition Reviews* **6,** *89, 90, 245 (1948)*; **7,** *255 (1949)*) are but a few of the many observations which might be offered in support of this idea.

A. E. Schaefer, W. D. Salmon, D. R. Strength, and D. H. Copeland (*J. Nutrition* **40,** *95 (1950)*) have been able to show that the nutritional requirements for folic acid, vitamin B_{12}, and choline are mutually interdependent in both rats and chicks under certain experimental conditions. Their experiments were conducted using a basal diet composed of extracted peanut meal, casein, cystine, sucrose, cod liver oil, lard, salts, and the usual vitamins except B_{12} and folic acid. This diet provided 0.3 per cent methionine and only 0.007 per cent choline. When the basal diet was supplemented with 0.04 per cent choline chloride or 0.128 per cent methionine and fed to weanling rats, 100 per cent of the animals developed renal hemorrhage within two weeks. Addition of 2, 10, or 100 mg. of folic acid per kilogram of the choline-supplemented diet reduced the incidence of renal hemorrhage to 75, 42, and 50 per cent, respectively, while 30 to 150 micrograms of vitamin B_{12} per kilogram of ration reduced the incidence 50 per cent. However, 10 mg. of folic acid with 30 micrograms of vitamin B_{12} completely prevented the renal lesions. Vitamin B_{12} and folic acid were without effect unless choline or methionine (presumably) were added to the ration. Both folic acid and B_{12} were effective in preventing death, in lessening the severity of renal hemorrhage, when it occurred, and in promoting growth. Vitamin B_{12} and folic acid together promoted slightly better growth than either alone.

When day-old chicks were fed the basal diet supplemented with 0.6 per cent choline chloride, rather poor growth occurred. Thirty micrograms of vitamin B_{12} or 2 mg. of folic acid per kilogram of ration caused growth increments of 67 to 70 g. per chick in four weeks. Increasing the B_{12} to 150 micrograms or the folic acid to 100 mg. produced but little better growth. However, 30 micrograms of B_{12} plus 2 mg. of folic acid caused a growth increase of 126 g. per chick.

When chicks were fed the basal diet supplemented with only 0.10 per cent choline chloride, similar growth responses to folic acid and B_{12} were observed except that 2 mg. of folic acid were but slightly effective whereas 100 mg. of this vitamin had a marked effect. Again B_{12} and folic acid together produced the best growth. As little as 5 micrograms of B_{12} per kilogram of ration produced a marked effect when 100 mg. of folic acid were in the ration. On the other hand, only 0.2 mg. of folic acid was required to produce marked growth improvement when optimal B_{12} was present.

Hemoglobin concentration was about 25 per cent below normal in chicks maintained on the basal choline-supplemented ration. Addition of either folic acid or B_{12} produced nearly normal hemoglobin values, while B_{12}

and folic acid together produced slightly superior results.

Perosis occurred in a large percentage of the chicks not receiving folic acid or B_{12}. Two milligrams per kilogram of folic acid provided definite but incomplete protection, while 100 mg. gave complete protection against perosis. Large amounts of B_{12} gave incomplete protection. Small amounts of B_{12} plus small amounts of folic acid gave complete protection. If the diet contained no added choline, perosis occurred in all chicks even though the diet was supplemented with folic acid, B_{12}, and methionine.

Feathering was also poor in these chicks and both B_{12} and folic acid were required to restore normal feathering. The livers of the deficient chicks were large in proportion to their body weight. Either B_{12} or folic acid alone reduced the liver:body weight ratio but the maximum effect was observed when these vitamins were used together. A similar effect was noted with the weight of the gall-bladders.

These experiments certainly seem to demonstrate an interrelation between folic acid, B_{12}, and choline. In both the rat and chick experiments it is to be noted that neither B_{12} nor folic acid had these effects unless a certain amount of choline, or in some instances methionine, was added to the diet. If a minimal amount of choline was in the diet, the change caused by these vitamins was similar, at least in many instances, to that which could have been produced by adding more choline. Thus it might be stated that B_{12} or folic acid "spared" choline. Also it should be noted that neither folic acid nor B_{12} alone had a complete effect although both together did give a complete effect. Thus it might be visualized that B_{12} and folic acid can each spare a certain amount of choline and when used together can spare a larger amount sufficient to prevent completely the abnormal phenomena being observed, indicating that the effects of these two vitamins are additive and that B_{12} is not acting by substituting for folic acid or vice versa, at least in so far as the choline-sparing action is concerned. It is not likely, nor is it claimed, that the only effect of folic acid and B_{12} in these animals was to spare choline. In some of the experiments the effect produced by folic acid and B_{12} was considerably greater than that recorded with the largest amount of choline alone. It may indicate that folic acid and B_{12} have interrelations which do not concern choline.

C. A. Nichol, L. S. Dietrich, C. A. Elvehjem, and E. B. Hart (*J. Nutrition* **39,** *287 (1949)*) have published the results of some experiments concerning folic acid and B_{12} interrelations in chicks. Their experiments were conducted using a purified diet different from that used by Schaefer *et al.* (*loc. cit.*). It was composed of casein, gelatin, sucrose, salts, soybean oil, cystine, and the usual vitamins including choline, 0.15 per cent, but excluding folic acid and B_{12}. It would be expected to be sufficient in methionine and choline so that choline interrelations should not have entered into their experiments. They did not investigate this point, however.

A group of chicks was fed the basal ration for nineteen days. The chicks were then divided into three groups: one remained on the basal diet as a control; the second group received 1 U.S.P. unit of liver extract per chick every other day by injection; and the third group received a B_{12} concentrate which provided 3 micrograms of vitamin B_{12} per 100 g. of ration. The control birds failed to grow but developed no unexpected symptoms otherwise. The chicks receiving liver extract or B_{12} gained considerably in body weight, about half of them developed a peculiar syndrome characterized by extended quivering wings, body tremors, difficulty in walking and in maintaining balance, and complete paralysis followed by death in two to four days. An injection of 50 micrograms of folic acid produced complete recovery for twelve to fourteen days when the symptoms reappeared. Folic acid was much more effective when injected than when given orally.

In a second series of experiments the effect of B_{12} and folic acid, singly and together, on body growth, hemoglobin production, and feathering was studied. Vitamin B_{12} caused a significant growth stimulation when added to the folic acid-deficient ration or when given by injection, even though more than half of the chicks developed the peculiar neuromuscular syndrome described above. Folic acid produced growth stimulation to a greater extent than did B_{12}. Folic acid and B_{12} used together caused greater growth than either alone. Folic acid produced a slightly greater hemoglobin concentration than was observed in the control animals. Vitamin B_{12} did not increase hemoglobin, actually chicks receiving this vitamin had slightly lower hemoglobin values than did the controls. However, this is probably not a valid comparison since these animals grew more than did the controls and therefore probably had a greater blood volume. Therefore it is possible that the total hemoglobin of these chicks was at least equal to the controls. Chicks on the basal diet feathered poorly. Vitamin B_{12} did not improve feathering although folic acid did.

There are obvious similarities and dissimilarities between the results of this study and those of Schaefer and co-workers. The different experimental conditions make any direct, detailed comparison impossible. However, it can be said that both groups have found conditions where it was possible to demonstrate an interrelation between folic acid and B_{12}, and, in some instances, a further relation to choline. Furthermore, the effects of folic acid and B_{12} seem to be additive, suggesting that each substance exerts a separate effect or produces the effect by a different mechanism. The relation of these vitamins to choline may explain a part, but only a part, of their effect.

In the present state of knowledge it is futile to speculate as to the biochemical mechanisms involved. They are undoubtedly complex and elucidation of them may be difficult. Further research will be necessary for a precise understanding of this important subject.

METABOLISM OF PHOSPHOLIPIDS WITHIN THE CYTOPLASM OF LIVER CELLS

The liver is importantly concerned in the metabolism of phospholipids. Although contributions are made to the pool of phospholipids circulating in plasma by intestinal absorption or synthesis (C. Artom and M. A. Swanson, *J. Biol. Chem.* **175,** *871 (1948)*), the principal source of plasma phospholipids is the liver (*Nutrition Reviews* **5,** *135 (1947)*). This organ is equally important in removal of phospholipids from plasma.

G. L. Ada (*Biochem. J.* **45,** *422 (1949)*) has attempted to determine the relative activity of various structural components of hepatic cells in the turnover of phospholipid in the liver. By methods previously described by A. Claude (*J. Exp. Med.* **84,** *51 (1946)*) and by G. H. Hogeboom, W. C. Schneider, and G. E. Pallade (*J. Biol. Chem.* **172,** *619 (1948)*) it is possible to separate a number of cellular structures. The tissue is macerated and fractionated from suspensions by the use of a high-speed centrifuge. Cell nuclei and intact cells are removed and three cytoplasmic fractions are obtained. The fractions include particles of varying size and density. The first fraction separated is composed of large granules comparable to mitachondria and secretory granules. The second contains particles too small to be visualized in the light-microscope. These are designated as microsomes. The third fraction includes cytoplasmic components remaining in suspension or in solution after separation of the first two fractions.

Ada obtained fractions from hepatic cells of rabbits using a modification of the original methods. The rabbits used were full-grown, and food was withheld for about twelve

hours before they were killed. No description is given of the diet previously provided these rabbits.

The total lipid, phospholipid, nucleic acid, and protein content of whole liver, and of large and small particles of the cytoplasm of liver cells were determined by chemical analysis. Concentration of total lipid and phospholipid was greater in large granules and microsomes than in whole liver. The total lipid content of the large granule fraction was variable. This was interpreted as indicating lack of homogeneity of this fraction, but attempts to achieve a homogenous product by further centrifugation were not successful. Small granules or microsomes contained more lipid than did the large granules and the concentration of total lipid in this fraction was relatively constant.

The electrophoretic mobility of the small granule fraction indicated particles of equal surface density charge. The amounts of phospholipid in the cytoplasm in the large particles, small particles, and the supernatant or third fraction were calculated to be 26.4, 64.6, and 9 per cent, respectively. The author believes that probably differences in lipid concentration and density, rather than particle size, determine separation by differential centrifugation.

Concentrations of nucleic acid and protein in large and small granules were variable but were somewhat higher in the small granule fraction. The total lipid, nucleic acid, and protein made up 94 per cent of the dry weight of large granules and about 96 per cent of the total solids of small granules.

The radioisotope of phosphorus, P^{32}, contained in sodium phosphate was injected into other rabbits in an experimental attempt to determine the relative activity of various fractions of cytoplasm in phospholipid synthesis. In one group of experiments radiophosphorus was administered by continuous intravenous infusion. By this means a constant level of inorganic P^{32} was maintained in the plasma for four hours. At the end of this period the animals were killed and the specific activities of plasma inorganic phosphorus, liver inorganic phosphorus, and phospholipid phosphorus in the nucleus and three cytoplasmic fractions of hepatic cells determined. The specific activity of liver inorganic phosphorus was found to be about one-half that of plasma. Uptake of the radioisotope in phospholipid was much less rapid. The specific activity of phospholipid phosphorus in the various cell fractions was greatest in microsomes followed in order by large granules, nuclei, and supernatant, respectively. Differences were not great, however. The specific activity of the small granules was 1.3, as compared with 0.7 in the supernatant. Indications were that phospholipid fractions of different specific activities are to be found in hepatic cells.

The author next set out to determine whether phospholipids of the various fractions are synthesized independently of each other, or whether those of the small granules with highest specific activity exist as precursors of phospholipids of other fractions. The data accumulated indicated that the phospholipid of no one of the liver components served as a precursor of the phospholipids of another fraction. There was evidence that the phospholipids of the large particles were precursors of plasma phospholipids and that under the conditions of these experiments, 25 to 30 per cent of the total phospholipid synthesized within the liver is released to the plasma, the remainder staying within the liver cells for purposes of cellular metabolism.

These investigations are intriguing and their results of importance. The methods utilized seem to add another dimension to metabolic investigations. By such means one can study physiologic reactions and their rates in fragments of cells. Identical or similar syntheses may occur in various parts of a cell but at different rates and apparently wholly unrelated to synthesis in another type of cell particle. Extension of these studies to determine production rates of different types of phospholipids, *i.e.*, lecithin, cephalin, and sphingomyelin in various cell fractions, would be of interest.

POTENTIAL INTERRELATIONS OF CHEWING ABILITY AND NUTRITURE

Earlier studies at the Nutrition Clinic of the Hillman Hospital in Birmingham, Alabama were concerned with the incidence of alimentary disturbances in a group of malnourished edentulous individuals (*Nutrition Reviews* **4,** *139 (1946)*). Of the 160 patients studied, 112 had disturbances of the digestive tract; all had a previous history or present evidence of chronic pellagra, beriberi, scurvy, and riboflavin deficiency.

The vertical dimension of the face was found to be less than normal in 140 of the 160 cases by reason of inadequate artificial dentures. One hundred and nine of these 140 cases had definite evidence of perlèche. In contrast, only 20 of those with a normal vertical dimension had any evidence of angular cheilosis. By reason of the inadequacy of the dentures in these subjects, they were unable to consume adequate amounts of a varied diet. Administration of B-complex vitamins resulted in a reduction in the extent of the cheilotic lesions but did not completely heal the lesions.

In a continuation of these studies, H. I. Greene, S. Dreizen, and T. D. Spies (*J. Am. Dent. Assn.* **39,** *561 (1949)*) made a clinical survey of the incidence of impaired masticatory function in 446 consecutive patients at the Nutrition Clinic of Hillman Hospital in Birmingham, Alabama. Masticatory insufficiency was a term used to describe a variable clinical condition which resulted in an inadequate ability to chew food. A patient was considered to be in a state of masticatory insufficiency if any of the following conditions prevailed: (1) no natural teeth and artificial dentures which were inadequate or painful; (2) no natural teeth and no artificial dentures or only one denture; or (3) less than three opposing, serviceable, natural, masticating teeth and three opposing, serviceable, natural, anterior teeth, and no functional replacements in the form of dentures or bridges.

The 446 patients ranged in age from 12 to 87 years; there were 340 women and 106 men. Sixty per cent (268) of these patients had no natural teeth; only 49 of these edentulous individuals had well-balanced, functional artificial dentures. Of the 178 individuals who still had some natural teeth, only 96 had well-balanced dentitions. Thus, 301 (67.8 per cent) of the 446 patients had various degrees of masticatory insufficiency and only 145 had natural or artificial, well-functioning dentitions. The incidence of masticatory insufficiency was observed to increase with age; for example, 58 or 92.1 per cent of the 63 patients in the age group between 66 and 70 years were in masticatory insufficiency.

In addition to examination of the teeth, gross appearance of the soft tissues of the oral cavity was studied and any abnormal findings recorded. A dietary and social history and a questionnaire concerning presence of alimentary tract disturbances completed the special examination. Such complaints as dry mouth, scalded tongue, sore mouth, anorexia, stomachaches, and constipation were recorded.

The data obtained from the interrogations regarding disturbances of the alimentary tract were summarized and appeared to indicate that there was a slightly higher incidence of subjective complaints of alimentary tract disturbances among the patients with masticatory insufficiency. Of the 301 patients with masticatory insufficiency, 255 (84.7 per cent) registered 559 complaints referable to the alimentary tract; in comparison, 107 (73.7 per cent) of the 145 with well-functioning dentures described a total of 230 similar complaints.

For each of the individual categories of complaints, the percentage of patients describing that disorder was slightly higher among the group with masticatory insufficiency, except for stomachaches where the incidence was slightly higher in the superior group. The greatest difference between the two groups was listed for the category of sore mouth where 31.5 per cent of the group

with masticatory insufficiency complained of this disorder in contrast to only 16.5 per cent of the group with well-functioning dentures.

From these data the investigators concluded, "These observations support our hypothesis that an intimate relation exists between impaired masticatory function (masticatory insufficiency) and general nutritive failure." This conclusion is difficult to justify on the basis of the data presented by the investigators.

The patients were a highly selected group by reason that they had presented themselves to the clinic for nutritional assistance. Thus, it would be expected that there would be a high incidence of alimentary complaints among both groups. Whether there is any significant difference between the incidence of any of these complaints is a debatable point. The two complaints with greatest differences in incidence were sore mouth and stomachaches. The latter was considerably more common in the group with well-functioning artificial or normal dentures. On the other hand, sore mouth was considerably more common among the patients which were considered to have a reduced masticatory function. The origin and exact description of this complaint was not defined in the article.

One of the most prevalent and best recognized causes of sore mouth is the type of lesion, or lesions, caused by badly fitted, loose, or broken dentures and by broken malplaced and unopposed natural teeth. Unquestionably in a group of 301 patients, numerous of the cases of sore mouth must have resulted from mechanical interference. Logically one would expect that such lesions would occur under less stress and abrasion in patients that were malnourished, but occurrence of sore mouth in the presence of mechanical injury cannot be interpreted to be purely nutritional or systemic in origin.

It seems entirely reasonable that the restriction in selection of foods necessitated by restricted masticatory function would tend to increase the tendency for inadequate nutriture. However, the data presented do not clearly demonstrate this relation.

A NUTRITION STUDY IN NIGERIA

In nutrition surveys the error has frequently been made of attaching diagnostic significance to a given clinical sign and then using the frequency of the sign to estimate the incidence of the supposed deficiency within the population. It is now apparent that no one clinical sign can yield a valid measure of a given deficiency within a population. Instead, the nutriture of a people is revealed only by consideration of many clinical findings in relation to dietary and/or laboratory data. Clinical information must also include knowledge of medical problems which might alter, result from, or simulate nutritional effects.

Recognition of these principles is well illustrated in a useful study of the nutrition of Nigerian farmers (B. M. Nicol, *Brit. J. Nutrition* **3**, *25* (*1949*)). This nutrition survey compared the clinical features of three groups of African peasant farmers of racial similarity, living within two hundred miles of each other under the same climatic conditions and consuming diets similar in staple foodstuffs, but varying in sources of carotene and riboflavin.

The study was made in Niger Province, a tropical area with a mean annual rainfall of between 40 and 60 inches. The altitude of the regions ranged from 500 to 1400 feet, but there was no significant variation in mean relative humidity. Thus, comparison of skin lesions within the groups was not complicated by climatic differences. The three districts included were Bida, where red oil palms are cultivated, and Kontagora

and Zuru where the palms do not grow and shea-nut oil and small quantities of butter replace the red palm oil as cooking fat. In Zuru, Guinea corn replaced bulrush millet and roots in the diet, and, hence the populace consumed more riboflavin.

All of the food consumed by three families in each area was weighed for four periods of seven days each during the year. Calculations were made of nutrient composition of the respective diets. The staple foodstuffs in all three areas included Guinea corn, bulrush millet, cassava, and sweet potatoes (especially in Bida). Dried-river fish was the main source of animal protein. The use of red palm oil for cooking was limited to Bida, a fact which accounted for the following great difference in the daily vitamin A intakes: Bida, 5174 I.U.; Kontagora, 625 I.U.; Zuru, 713 I.U. The variation in grain consumption described above resulted in a daily intake of riboflavin in Zuru of 0.98 mg. in contrast to 0.76 mg. in each of the other districts. The protein intake was highest in Zuru.

Clinical evaluations were made of the nutritional status of the individuals whose food intake had been examined and of "as many of the neighboring farmers and their families as possible." The total sample amounted to 264 subjects, similarly distributed between the districts as to age and sex. Numerous clinical features were recorded, with particular attention to the skin, eyes, lips, tongue, and gums. Clinical evidence of liver disease was carefully noted. In all instances the clinical features were described rather than assigned a given significance.

The investigator has grouped the clinical observations according to their difference or similarity of incidence from district to district. The following signs occurred in the three areas with equal frequency: folliculosis, ulcers and scars of ulcers, atrophic skin, conjunctival thickenings, pigmentation of the sclera, photophobia, color changes in the tongue, fissuring of the tongue, and gingivitis. It would seem, therefore, that these changes are unrelated to the differences in dietary intakes in vitamin A, riboflavin, and protein of the magnitude recorded. It is of especial interest that conjunctival changes occurred with equal frequency in these groups with such widely differing intakes of vitamin A. Morphologic changes in the tongue were frequent despite a rather high intake of niacin (19 to 25 mg. daily).

The following findings occurred with significantly ($P < 0.03$) less frequency in Bida (region of higher carotene intake) than in the other two districts: dry, staring hair and achromotrichia, grade 2 xerosis, follicular hyperkeratosis, grades 2 and 3 of "elephant skin," crackled skin, night blindness, and corneal opacities. It would seem reasonable to feel that these changes are at least in part related to the vitamin A intake. In Zuru (district of highest riboflavin intake) there was significantly less angular stomatitis, nasolabial seborrhea, permanent gooseflesh, and conjunctival injection. These features, then, may reasonably be considered as evidences associated with differences in consumption of riboflavin and/or protein.

The skin change of folliculosis deserves comment. Nicol has divided the skin lesions sometimes grouped under this term into two types: folliculosis (keratosis pilaris), "a simple enlargement or undue prominence of hair follicles, without obvious hyperkeratosis, which may occur on either a healthy-looking or a xerotic skin"; and follicular hyperkeratosis (phrynoderma) "marked thickening of hair follicles with projecting spiny plugs of keratinized material, occurring superimposed upon a generalized xerosis." The former was not epidemiologically associated with vitamin A deficiency; the latter seems to be related to avitaminosis A as it was not observed in the district of Bida. The relation of this sign to avitaminosis A has been debated in recent years. S. B. Wolbach and O. A. Bessey (*Physiol. Rev.* **22,** *233 (1942)*) suggested that the lesion might be related to ariboflavinosis. Nicol's findings do not seem to support this sug-

gestion since no follicular hyperkeratosis was observed in the district in which the riboflavin intake was low and the vitamin A intake high. It may be, of course, that a low intake of both vitamins is required to produce the lesion.

C. Gopalan (*Indian Med. Gaz.* **82,** *16 (1947)*) studied 116 cases of phrynoderma in India and observed the effect of various therapeutic agents including vitamin A concentrate, niacin, riboflavin, yeast extract, linseed oil, and yeast extract plus linseed oil. No definite or complete therapeutic response resulted from treatment with the first three agents. Good responses following yeast extract and/or linseed oil were noted. Gopalan suggested that ". . .phrynoderma may be due to related deficiencies of certain members of the B_2 complex and fatty acids." Such a hypothesis would not be inconsistent with the Nigerian study since the populace of the district with a high-vitamin A intake received the vitamin (carotene) in palm oil. It is regrettable that laboratory findings are not available in this study to aid in elucidation of the problem.

The importance of ancillary clinical studies in interpretation of nutrition survey findings is well illustrated by Nicol's observations that all 3 cases of night blindness which occurred in the district of Bida where the vitamin A intake was high and an appreciable proportion of those from other areas had liver disease. Furthermore, in all three districts almost all of the subjects exhibiting corneal opacities had some degree of liver damage. This correlation was so high that Nicol expressed doubt whether vitamin A deficiency could be considered the primary cause of the corneal lesions. Such thoughtful medical appraisal of the results of nutrition surveys will lead to better understanding of the true role of nutrition in health and disease. To be further commended in this survey is the planning which made possible comparison of data from similar areas with differing intake levels, a circumstance which makes the findings more meaningful than had a similar number of persons been examined in one population alone.

INVESTIGATIONS OF NUTRITIONAL STATUS IN GREAT BRITAIN

The topics discussed at the forty-fifth scientific meeting of the British Nutrition Society fell into two categories: (1) appraisal of methods for assessment of nutriture, and (2) results of investigations on the nutritional status in Great Britain.

In the first group, which is disproportionately large, J. Yudkin (*Brit. J. Nutrition* **2,** *158 (1948)*) evaluated anthropometric and performance tests. His paper was commented upon by E. R. Bransby (*Ibid.* **2,** *171 (1948)*). The correlation (or lack of it) between results of clinical surveys on one hand and biochemical, somatometric, and performance measurement was discussed at some length by H. M. Sinclair (*Ibid.* **2,** *161 (1948)*). C. C. Ungley (*Ibid.* **2,** *170 (1948)*) exemplified the difficulties met in assessment of nutritional status by analyzing a case of edema.

The laboratory (biochemical) approach to the characterization of the state of nutrition was discussed by J. R. Marrack (*Brit. J. Nutrition* **2,** *147 (1948)*). The laboratory methods, as contrasted with clinical appraisal, supply impersonal data, free of temperamental bias. Their meaning is the same the world over. The reliability of the methods can be determined and, by careful analysis of the procedures, it can be improved. British work on methods for determination of hemoglobin levels in the blood is cited as example.

Marrack (*loc. cit.*) reviewed, in some detail, British investigations on hemoglobin levels, ascorbic acid, vitamin A, vitamin D, and serum protein content as indicators

of the state of nutriture. The hemoglobin values in 1944 to 1946 were higher than in 1941 to 1942. The most numerous data available for documentation of this trend were obtained for pregnant women. The proportion of women, included in the survey, supplementing their diets with iron was not known. Before the war, fresh oranges and canned fruit helped to counteract the decrement in the intake of ascorbic acid resulting from the fact that in spring and early summer green vegetables are scarce in England and the amount of ascorbic acid in potatoes is lowest.

During wartime the loss of citrus fruits was reflected by marked seasonal trends in the results of saturation tests. Before the war, the average number of days necessary for saturation (method of L. J. Harris and M. A. Abbasy, *Lancet* **II,** *1429* (*1937*)) in boys, 8 to 14 years of age, was one and a half, while in the spring of 1941 it rose to three and a half. It cannot be determined whether health and efficiency had suffered from the lowered intake of ascorbic acid. At any rate, there was no risk of scurvy.

Several reports are available on vitamin A nutrition. The picture is not as clear as in the case of ascorbic acid. In 1946 and 1947 Marrack found, in the livers of newborn babies, concentrations of vitamin A as high as those reported from the United States. The values are much higher than those obtained in England by J. B. Ellison and T. Moore (*Biochem. J.* **31,** *165* (*1937*)). Little information suitable for comparative studies is available on inorganic phosphate and phosphatase in the plasma of infants and children.

A study on the nutritive value of human milk in relation to the diet was reported by S. K. Kon and E. H. Mawson (*Proc. Roy. Soc. Med.* **40,** *900* (*1947*)). Vitamin A did not vary with the amount supplied by the diet. On the other hand, ascorbic acid paralleled rather closely the intake, with a minimum in the spring and a rise when new potatoes became available. The amount of thiamine in the milk increased after milling of flour to 85 per cent extraction was introduced as a compulsory measure in 1942.

Marrack cites a study by A. C. Chibnall, M. W. Rees, and E. F. Williams (*Biochem. J.* **37,** *354* (*1943*)), demonstrating the inadequacies of the methods used for estimating the serum protein content. Furthermore, he expresses doubt about the validity of the concentration of protein in the serum as an index of protein nutrition. No conclusion as to the adequacy of the protein, especially animal protein, in the British diet was reached.

EFFECT OF FAT ON THE EMPTYING TIME OF THE STOMACH IN FIBROSIS OF THE PANCREAS

Emptying of the stomach is delayed when fat is fed. It has been shown that gastric motility and tone decrease, and that the pylorus closes when fat fed the human subject reaches the upper portion of the duodenum. These effects occur almost immediately if the fat meal is placed in the duodenum. Introducing the fat lower in the intestine fails to elicit this response or makes it more difficult to demonstrate.

The effect of fat on the stomach and pylorus has been described as the "duodenal mechanism" (J. Gershon-Cohen, *Radiology* **48,** *232* (*1947*)). Earlier experiments had shown that an extract prepared from duodenal mucosa which had previously been in contact with fat inhibited gastric activity in experimental animals. It was postulated that a humoral mechanism was responsible. The active principle has been prepared in relatively great concentration and has been called enterogastrone (J. S. Gray, W. B. Bradley, and A. C. Ivy, *Am. J. Physiol.* **118,** *463* (*1937*)).

Patients with fibrosis of the pancreas and with resulting lack of pancreatic secretions in the intestine have been observed to have voracious appetites which abate when dried pancreas is fed (C. D. May and C. U. Lowe, *Pediatrics* **1,** *159 (1948)*). Roentgenographic studies of the stomach and intestine were undertaken seeking acceptable physiologic explanations of the ravenous appetite of these patients. A second purpose was to determine whether the pancreas is involved in elicitation of the "duodenal mechanism" or in the production of an enterogastrone-like substance (Lowe, May, H. M. Stauffer, and E. D. B. Neuhauser, *Am. J. Dis. Child.* **79,** *91 (1950)*).

One to two hundred milliliters of barium-sulfate suspension were administered by mouth to the children who served as experimental subjects. By fluoroscopic examination the moment of passage of the barium into the duodenum was determined. At intervals of fifteen, thirty, forty-five, and sixty minutes, thereafter, roentgenograms of the abdomen were taken. In normal subjects there was passage of considerable quantities of barium from the stomach within fifteen minutes, and at the end of an hour about 0 per cent of the dose administered remained in the stomach. The barium was evenly distributed through the duodenum, jejunum, and upper ileum. Three to five days later the same subjects were again given a similar quantity of barium-sulfate suspension to which corn oil was added to a content of 30 per cent. After initial ejection of a small quantity of barium into the duodenum there was delay in gastric emptying, and after an hour little barium could be seen in the small intestine. That which had passed the pylorus clumped in intestinal loops, producing a picture similar to the deficiency pattern of steatorrhea or nutritional disease.

When the same procedures were applied to children with fibrosis of the pancreas, gastric emptying and distribution of barium through the small intestine were similar to those observed in normal subjects when fat was not added to the barium suspension. However, administration of the fat meal uniformly produced a picture unlike that observed in control subjects. Instead of a delay in gastric emptying, there was a tendency for even more rapid passage from the stomach. Also, there was even distribution of the barium in the intestine and no clumping.

Roentgenographic observations were repeated in 2 children with fibrosis of the pancreas thirty minutes after oral administration of 3 g. of pancreatin. Under these conditions there was delayed gastric emptying after the fat meal. No more than 10 per cent of the barium left the stomach within a period of one hour. There was clumping in the loops of small bowel, producing a pattern comparable to that seen in normal subjects following the meal containing corn oil.

Results of these experiments indicate that the delay in gastric emptying and clumping of contents in the small intestine associated with a fatty meal in normal individuals does not occur in patients with fibrosis of the pancreas. Administration of pancreatin seems to correct the defect in gastric and intestinal motility in these patients. Failure of fat in the food consumed to initiate the "duodenal mechanism" may contribute importantly to the increased appetite associated with this disease. It is of interest that the appetite is decreased when pancreatin is fed.

The suggestion is offered that the "duodenal mechanism" or the humoral agent responsible for it depends either upon normal pancreatic function or the products of pancreatic digestion. It is suggested, also, that the clumping intestinal pattern associated with steatorrhea is the result of the normal response to fat in the intestine, since it would be anticipated that patients with this disorder would have some residual fat in the intestine at all times. However, it should

be pointed out that clumping or segmentation of barium sulfate in the intestine has been observed in normal infants (R. Golden, *Radiology* **36,** *262* (*1941*)), and has been induced by addition of hypertonic solutions as well as fat to the barium sulfate. Mucous secretions flocculate the suspension of barium. It is possible that fatty acids and hypertonic solutions produce their effect by stimulation of these secretions (A. C. Frazer, J. M. French, and M. D. Thompson, *Brit. J. Radiol.* **22,** *123* (*1949*)).

The patient with fibrosis of the pancreas provides an important experimental tool, making possible further investigation of the exocrine functions of the pancreas. It is anticipated that other studies of the type reported here will be undertaken.

PREFERENTIAL INACTIVATION OF OXIDIZED PHOSPHOPYRIDINE NUCLEOTIDES IN TISSUES

Studies of the metabolism of the di- and triphosphopyridine nucleotides (DPN and TPN) have indicated that these coenzymes, frequently designated coenzymes I and II, undergo constant synthesis and degradation, and that the actual level of tissue coenzyme available for tissue oxidations (hydrogen transport) is the resultant of these two processes (*Nutrition Reviews* **8,** *48* (*1950*)).

H. McIlwain (*Proc. Roy. Soc.* (*London*) *B* **136,** *12* (*1949*); *Nutrition Reviews, loc. cit.*) has shown further that a part of coenzyme I (DPN) catabolism in *Lactobacillus arabinosus* is directly associated with its activity as a co-factor in glycolysis. McIlwain and R. Rodnight (*Biochem. J.* **44,** *470* (*1949*); **45,** *337* (*1949*)) have now shown that another enzyme system concerned with the degradation or breakdown of the phosphopyridine nucleotides is affected by the functional state of these coenzymes.

P. Handler and J. R. Klein (*J. Biol. Chem.* **143,** *49* (*1942*)) demonstrated the existence of an enzyme system in a variety of minced tissues which caused fission of diphosphopyridine nucleotide at the pyridinium nitrogen with a liberation of niacinamide. McIlwain and Rodnight have studied the action of this enzyme upon oxidized and reduced DPN and TPN and certain other related compounds. The sources of enzyme used by these workers were tissue suspensions and purified extracts of brain and spinal cord from rats, rabbits, guinea pigs, and sheep. Since the cleavage of coenzyme in this system results in conversion of the quarternary nitrogen of the pyridinium salt of coenzyme into the tertiary nitrogen of free niacinamide with liberation of a mole of acid, it was possible to follow the rate of reaction by observing the rate of carbon-dioxide evolution from a bicarbonate buffer. Free niacinamide was also determined at the end of the reaction. The enzymatic activity responsible for the breakdown of DPN and TPN remained in the tissue particles after thorough washing with isotonic solutions and could be extracted from these washed particles by water or strong salt solutions. The enzyme was relatively stable at 37°C. but was inactivated at 70°C. Addition of niacinamide markedly inhibited the rate of reaction.

Both DPN and TPN were rapidly inactivated by this enzyme, TPN being inactivated at a rate almost equivalent to (80 per cent) that of DPN. The fact that these two coenzymes competed for inactivation when present together made it evident that a common enzyme was involved. Of great interest was the finding that reduced DPN and TPN ($DPNH_2$ and $TPNH_2$) were not inactivated by this system. Neither did the reduced coenzymes affect the breakdown of the oxidized forms when added together. McIlwain and Rodnight believe that the inertness of the reduced forms in

this system is due to the shift in the valence of the pyridinium nitrogen atom upon reduction from a quarternary to a tertiary group. It appears that the enzyme is specific for an ionized quarternary nitrogen group. For activity, the enzyme also requires a substrate with at least a mononucleotide structure since niacinamide ribofuranoside (the vitamin-ribose component of DPN and TPN), niacinamide glucoside, niacinamide mannoside, and niacinamide galactoside were not inactivated.

The effects upon tissue metabolism of this differential attack upon oxidized but not reduced coenzymes I and II may be extensive and undoubtedly require further investigation. The destruction of one member of an oxidation-reduction system, for example, should markedly alter the oxidation-reduction potential within the cell and effect other oxidation-reduction systems sensitive to such changes in potential. The fact that certain agents which inhibit the Pasteur effect also inhibit DPN destruction (H. McIlwain, *Biochem. J.* **44,** *xxxiii* (*1949*)) also suggests that the enzyme system degrading the pyridine nucleotides may have a central role in tissue metabolism.

PANTOTHENIC ACID DEFICIENCY IN RATS

Pantothenic acid deficiency is readily produced in a variety of young, growing animals. In the rat, symptoms include, besides growth failure, hemorrhage and necrosis of the adrenals, anemia, and leukopenia, porphyrin-staining of the fur and whiskers, and if black rats are used, a rather striking graying of the fur (*Nutrition Reviews* **4,** *163* (*1946*)). In adult animals, however, induction of obvious signs of pantothenic acid deficiency, as well as of many other vitamin deficiencies, becomes a long and tedious procedure. In the absence of the heightened demands for growth, depletion appears to be a slow process.

Within recent years, various investigators (*cf.* B. H. Ershoff, *Physiol. Rev.* **28,** *107* (*1948*)) have emphasized that the bodily requirement for essential nutrients can be increased by any of a number of so-called "stress factors" which include, for example, hyperthyroidism, fevers, pregnancy, lactation, certain drugs, toxins, etc. Growth itself might be looked upon as a "stress factor," especially when induced under conditions where it would not otherwise be present. This is possible in adult animals by injection of the growth hormone.

A demonstration that under such conditions, sensitivity to a dietary lack of pantothenic acid is increased has been made by W. D. Lotspeich (*Proc. Soc. Exp. Biol. Med.* **73,** *85* (*1950*)). Adult female rats received a pantothenic acid-free ration high in either carbohydrate or fat. In a preliminary two-week period on these rations, no growth was observed. During a subsequent sixteen-day period, a daily intraperitoneal injection of 1.0 mg. of crystalline anterior pituitary hormone was given. A growth response was noted within one day which continued for about one week. At this time, all animals began to lose weight and to show porphyrin encrustation of the whiskers, bloody nares, and other symptoms of pantothenic acid deficiency. Upon administration of pantothenic acid, spectacular growth gains were recorded, and all symptoms disappeared. Thus a rapid establishment of uncomplicated pantothenic acid deficiency in adult animals followed induction of growth through use of the growth hormone. Although not unexpected, the result constitutes a nice demonstration of the enhancement in bodily requirements for a nutritive essential induced by a resumption in growth.

VITAMIN E DETERMINATION IN BLOOD AND TISSUES

The development of reliable microchemical methods for determining the concentration of various nutritive essentials, or their corresponding tissue derivatives, in blood has made it possible to report specific laboratory findings in nutrition surveys. The microprocedures for vitamin A, carotene, ascorbic acid, riboflavin, serum protein, hemoglobin, and alkaline phosphatase have been reviewed (*Nutrition Reviews* **4,** *141 (1946)*). In the case of vitamin E determination, chemical procedures must be able in the presence of interfering substances to evaluate the distribution of tocopherols in terms of the individual tocopherols and to estimate the over-all biologic activity to be expected from the total tocopherol content.

M. L. Quaife, N. S. Scrimshaw, and O. H. Lowry (*J. Biol. Chem.* **180,** *1229 (1949)*) have presented a micromethod for measuring the amount of vitamin E in blood. The method is based on the macroprocedure of Quaife and P. L. Harris (*Ibid.* **156,** *499 (1944)*) which has been refined and modified to the point where only 0.06 ml. of serum or plasma is required. The instrument used to measure the optical density of very small volumes was the adaptation of the Beckman spectrophotometer which has been described (*Nutrition Reviews, loc. cit.*).

The A. Emmerie and C. Engel (*Rec. d. trav. chem. d. Pays-Bas* **58,** *283 (1939)*) color reaction was developed with ferric chloride and α,α' dipyridyl to give a red color. The light absorption, due to this color reaction, was measured at 520 mμ. Since the carotenoids interfere in this reaction, the light absorption due to carotenoids, prior to color development with α,α' dipyridyl, was measured at 460 mμ. A correction was then deducted from the absorption value determined at 520 mμ to give a true reading for the tocopherols. Using crystalline materials, it was found that carotene gave a reading at 520 mμ with the Emmerie-Engel reaction which is 29 per cent of that of carotene *per se* at 400 mμ.

A series of eighteen samples of human blood serum was used to compare results obtained with the macromethod and the micromethod. The results with the micromethod agreed reasonably well with those of the macromethod: eleven of the eighteen comparisons varied 5 per cent or less, and only three of the eighteen comparisons varied by more than 10 per cent.

M. L. Quaife and P. L. Harris (*Anal. Chem.* **20,** *1221 (1948)*) described a chemical assay for the vitamin E content of foods. The method consisted of freezing and dehydration of the food, homogenization of the sample by mechanical grinding of the sample while frozen, extraction of tocopherols and lipids, molecular distillation of the extract, hydrogenation of the distillate, and colorimetric analysis for tocopherols in aliquots of the hydrogenated distillate. Total tocopherols were measured by the procedure of Emmerie-Engel color reaction (*loc. cit.*) as developed by Quaife and Harris (*loc. cit.*). Because beta-tocopherol has been demonstrated only in wheat germ oil, the value determined by this procedure actually represented the total of alpha-, gamma-, and delta-tocopherols in foods other than wheat and wheat products.

The diazo method of M. L. Quaife (*J. Am. Chem. Soc.* **66,** *308 (1944)*) as modified by L. Weisler, C. D. Robeson, and J. G. Baxter (*Anal. Chem.* **19,** *606 (1947)*) was used to measure the total amount of gamma- and delta-tocopherols present. Since delta-tocopherol has been isolated only from soybean oil up until now, this value, found by the diazo method, probably signified the gamma-tocopherol content. Thus, the difference between the total tocopherol value and the amount of gamma- and delta-tocopherols gave approximately the concentration of alpha-tocopherol.

A modification of the above procedure which can be used for chemical estimation of vitamin E in animal tissues has been described by M. L. Quaife and M. Y. Dju (*J. Biol. Chem.* **180,** *263 (1949)*). Instead of the frozen dehydration and homogenization by mechanical grinding used for plant materials, the high-fat content and low-water content of the animal tissues necessitated preparation by freezing in liquid nitrogen and grinding in a mortar. Samples were then weighed into a Soxhlet thimble. The selection of an aliquot by this method was less satisfactory than that used for plant materials. Tocopherols were extracted in the fat fraction by exhaustive treatment with hot ethanol. Tests indicated that complete extraction was achieved within twenty-four hours. After extraction, total tocopherol content of the tissues was determined by the Emmerie-Engel color reaction (*loc. cit.*). The concentration of gamma- plus delta-tocopherol was determined by the diazo method referred to above. Recoveries were found to be satisfactory provided the tissues were fresh and unoxidized. Where rancidity had occurred, recovery values were low.

Similarly, tissues from freshly killed animals which had been maintained in a vitamin E-deficient state for prolonged periods gave low-recovery values. This procedure was used for determination of total tocopherol content of various human organs. These tissues were freshly obtained from a male and a female accident case. Both were apparently normal individuals. From these values and average organ weights recorded in anatomical textbooks, the approximate amounts of tocopherols in the entire organ were estimated. Body fat was found to be the major storage site for vitamin E in both subjects. The total amount of tocopherols in a woman weighing 40 kg. was calculated to be 6.560 g., while that of a man weighing 47 kg. was 2.309 g. More than 75 per cent of the tocopherol content in the female subject, and more than 50 per cent for the male subject, was found in the storage fat. About 4 per cent of the tocopherol content of the female was in the muscles, while about 12 per cent was present in the muscles of the male subject. The total tocopherol content of various organs in the male subject expressed as milligrams of tocopherol per 100 g. tissue varied from a low of 0.62 for abdominal muscle and 0.80 for kidney tissue up to a high of 29.2 for abdominal subcutaneous fat. When the total tocopherol values for the male were expressed on the basis of milligrams of tocopherol per gram of fat in the tissue, kidney tissue had the lowest concentration of 0.122 and testis the highest at 1.21. The total tocopherol content in the female expressed as milligrams per 100 g. tissue was observed to be lowest in the heart at 1.28 and in the uterus at 1.47, and highest in the abdominal subcutaneous fat 49.5. When the total tocopherol contents for the female were expressed on the basis of milligrams per gram of fat in the tissue, the lowest concentration was 0.257 for abdominal muscle and the highest was 1.16 for uterus. Alpha-tocopherol was the predominant form found in human tissues. In the male, the presence of gamma-or delta-tocopherol could be demonstrated only in the subcutaneous fat. However, gamma- or delta-tocopherols were present in appreciable amounts in most of the tissues of the female subject to the extent of 20 or 40 per cent of the total tocopherols. The identity of these materials was established by comparison of the diazotized dianisidine-coupling products of the tissue extracts with those of pure gamma- and delta-tocopherol.

These studies on the tocopherol content of human tissues constitute an interesting addition to knowledge of the distribution of tocopherols throughout the body. Probably the most interesting point is the demonstration of appreciable amounts of gamma- and delta-tocopherol in the female and their absence in the male, except in the subcutaneous fat.

NOTES

American Board of Nutrition

A certifying board, the American Board of Nutrition, to establish standards of qualification of persons as specialists in human nutrition and to certify as such persons who comply with the standards, has been established by persons and organizations interested in the practice of the science of nutrition. Those concerned with establishment of the Board include representatives from the American Institute of Nutrition, American Medical Association, The American Public Health Association, American Society of Biological Chemists, Inc., and Association of American Medical Colleges.

Present members of the Board are Otto A. Bessey, C. A. Elvehjem, Wendell H. Griffith, C. G. King, L. A. Maynard, Fredrick J. Stare, and John B. Youmans. Requirements for admission to examination and certification are as follows: (1) citizenship in the United States or Canada; (2) presentation of evidence of satisfactory moral and ethical standing; (3) Ph.D. in a biological science or M.D. degree from an approved school; (4) courses or training equivalent to a minimum of three-semester hours in at least three of the following five subjects: food technology, microbiology of food, human physiology, large-scale food preparation, and hospital dietetics; (5) three years' experience in the practical aspects of human nutrition.

Qualified candidates will be considered for certification without examination until June 30, 1951. Prescribed forms for applications, and further information may be obtained from the office of the Secretary-Treasurer, Otto A. Bessey, University of Illinois College of Medicine, 1853 W. Polk Street, Chicago 12, Illinois.

Ketogenic Action Of High-Niacin Diets In Rats

The only known catalytic activities of niacin in cellular metabolism result from its incorporation into di- and triphosphopyridine nucleotides (coenzymes I and II), which serve as essential coenzymes for a variety of dehydrogenation reactions. Such reactions occur continuously during normal catabolism of fats, carbohydrates, and proteins. There is no evidence that the number or speed of such reactions is enhanced in any way by an oversupply of the vitamin.

That huge doses of niacin can influence metabolism in an abnormal direction is indicated by the work of R. G. Janes and L. Myers (*Proc. Soc. Exp. Biol. Med.* **63,** *410 (1946)*). These workers reported that in rats made diabetic by administration of alloxan, administration of large amounts of niacin (1 g. per kilogram of diet) resulted in markedly increased excretion of acetone bodies in the urine (ketonuria) one to five days after initiation of the high-niacin ration. The severity and duration of this ketosis varied from animal to animal. It could not be ascribed to variation in the food intake, or to any factor other than the increased niacin intake. No indications of an altered carbohydrate metabolism accompanied the ketosis.

Subsequently, attempts were made to produce ketonuria in normal rats by niacin administration (R. G. Janes and J. Brady, *Am. J. Physiol.* **159,** *547 (1949)*). Nonfasting and fasting rats fed a basal synthetic diet were injected with 35 mg. of niacin per day over a five-day period. Urines were collected at twenty-four-hour intervals and acetone bodies determined. The nonfasting rats showed no increases in urinary acetone bodies following the niacin injections. However, large increases in such substances occurred in the niacin-injected, fasting animals but not in the fasted controls. When the niacin injections were discontinued, ketonuria disappeared, usually within two days, even though fasting was continued.

No explanation of these effects is presented by the authors. Because of the vasodilatory properties of amounts of niacin of

this magnitude, injections of two vasodilatory compounds, prostigmine and mecholyl, were made in fasting animals without producing ketonuria. It must be presumed that these unphysiologically high amounts of niacin cause, for reasons still unknown, either overproduction or underutilization of acetone bodies.

Gordon Research Conferences

The Gordon Research Conferences, sponsored by the American Association for the Advancement of Science, will be held again this year from June 26 to September 1 at the Colby Junior College, New London, New Hampshire.

Dr. E. L. R. Stokstad will be Chairman and Dr. W. J. Darby, Vice-Chairman of the Vitamins and Metabolism Conference, August 7 through August 11.

These Conferences were established to stimulate research in universities, research foundations, and industrial laboratories. It is achieved by an informal type of meeting consisting of scheduled lectures and free-discussion groups.

Requests for attendance at the Conferences, or for any additional information, should be addressed to W. George Parks, Director, Department of Chemistry, Rhode Island State College, Kingston, Rhode Island. From June 20 to September 1, 1950, mail should be addressed to Colby Junior College, New London, New Hampshire.

Nutrition and Fertility

The proceedings of the meeting of the Nutrition Society (J. Hammond, *Brit. J. Nutrition* **3**, *79 (1949)*) on nutrition and fertility will be of interest to many workers in the United States. The discussion considers the physiology of reproduction in relation to nutrition, spermatogenesis, and male fertility and nutrition, female fertility and nutrition, nutrition and viable young, and nutrition and human fertility.

Recent Nutrition Books

A Story of Nutritional Research. The Effect of Some Dietary Factors on Bones and the Nervous System. Sir Edward Mellanby. Published for Vanderbilt University by The Williams & Wilkins Company, Baltimore. 1950. Pp. 454. Price: $5.00.

People, Food, Machines. Stephen Raushenbush. The Public Affairs Institute, 312 Pennsylvania Ave., S.E., Washington 3, D. C. 1950. Pp. 80. Price: 50¢.